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"Point of Injury" Sampling Technology for Battlefield Molecular Diagnostics

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DARPA SBIR PHASE I FINAL REPORT: Point of Injury, Sampling Technology for Battlefield Molecular Diagnostics. W31P4Q-11-C-0222 (UNCLASSIFIED)

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Report Covers: April 25th through October 22nd

Executive summary

Technology

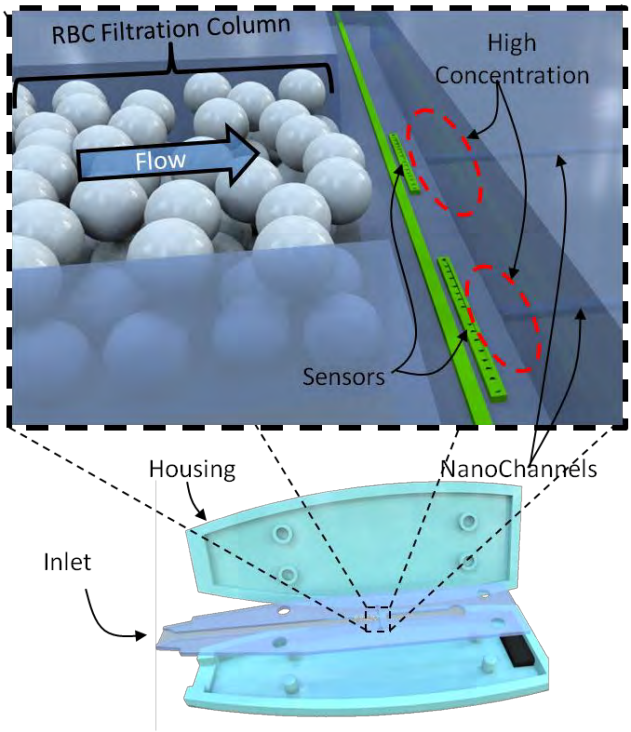
- The overall aim of this project is to develop a diagnostic field test that can be used to detect traumatic brain injury and late stage shock from a drop of blood taken from a finger stick.
- For Phase I, the goal is to develop the sample processing technology that takes blood from a finger stick and produces purified and concentrated plasma, within which we can detect rare blood-borne biomarkers.
- The technology should enable a portable system that can be set up in a remote location and enable clinical diagnosis of a variety of conditions. The system will be accompanied with disposable cassettes that are specific to the test.

Uniquenes

- The device has no moving parts, is made of durable material and should have a final footprint similar to a USB thumbdrive.
- The device consists of two stages. The first takes raw blood from a finger stick and produces filtered plasma. The second takes filtered plasma and produces a preconcentrated protein sample.
- The processing steps are not limited to a particular analyte, and the proposed sensing mechanism is multiplexable, allowing for a variety of diagnostics utilizing panels of markers.

Accomplishments

- Task 1: The blood filtration module has been completely developed and tested. This removes the enormous quantity of cells from the sample and provides clean plasma that can be diagnosed.
- Task 2: The concentrator device fabrication and chemistry testing is complete. We have achieved ~14 fold concentration in buffer and 1.6 times improvement in plasma.
- Task 3: Complete mechanical integration has been successfully developed. Final testing of the device was carried out. However leaks due to bond integrity failure prevented the final measurements using fresh whole blood spiked with our model label. These problems will be solved in Phase II.
- Extra: We have developed a working device packaging to improve ruggedness and sample delivery



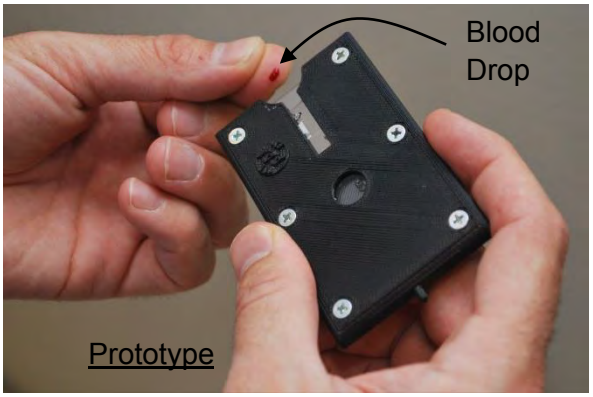


Figure 1: Overview of low power "molecular medic" system showing (left) a conceptual schematic showing an expanded view of the major components as well as (right) an image of the actual device inside ruggedized packaging. We use a simple multistage packed bead column followed by the "nanofluidic collapse" based molecular concentration method to ensure maximum interaction of the sample with the sensor. The sensor array can be used for multiplexed detection of a panel of biomarkers and control proteins.

1. Summary Statement

In this project Optofluidics, Inc. proposes to develop a "CLIA waivable" sample collection method for the concentration and quantification of blood-borne biomarkers associated with late phase hemorrhagic shock and traumatic brain injury. The overall goal for the phase I SBIR project is to develop an unpowered upstream sampling and processing technology for a point of injury diagnostic blood test. A conceptual overview is shown in Figure 1. This device will take raw blood and produce filtered, preconcentrated plasma that is ready for detection. As described in the proposal, the "technology we will develop in this SBIR is based on (1) a finger prick based assay, (2) a multistage packed bead filtration system, and (3) a protein concentration technique relying on nanochannels that are fabricated with a novel "nanofluidic collapse" technique originally demonstrated by the Erickson lab at Cornell University, and recently published in the Proceedings of the National Academy of Sciences." It is proposed that this sample collection technique can be integrated with our existing Nanoscale Optofluidic Sensor Array technology to create a handheld "molecular medic" that can rapidly detect the presence of biomarkers related to these conditions. ***Our goal is to provide our devices to untrained personnel or first responders who could diagnose with better certainty the presence of these injuries and make more informed decisions regarding treatment, having a dramatic influence on outcomes following a traumatic event.***

The original Phase I tasks are summarized below along with a brief description of our progress to date. In addition to the Phase I tasks, we have carried out additional work not described in the original proposal. This work consisted of the development of chip packaging to aid in device ruggedness and sample delivery. The packaged chip can be seen in Figures 1(right) and 11.

Task 1: Protein concentration system: This is accomplished by flowing the filtered plasma through nanochannels which cause a protein "traffic jam" at the channel entrance. The subtasks consisted of fabrication, specific detection of a protein in buffer and specific detection of protein in plasma. **This subcomponent has been successfully fabricated. The functionalization scheme has been fully developed and undergone full testing. Specific capture of streptavidin was increased an order of magnitude using the concentrator in buffer and 1.6 times in serum.**

Task 2: Blood filtration: After the blood enters the device via capillary action, the blood is filtered by a column composed of silica beads. The first subtask was to fabricate the device and the second was to perform testing on blood samples. **This stage of the device has been fully developed and tested with fresh blood taken directly from a finger stick without adding anticoagulation agents.**

Task 3: Integration: The final task consisted of integrating the blood filtration system with the protein concentration system and carrying out full-scale testing using spiked raw blood samples. **Device fabrication and integration of the components as well as a method for chemical functionalization of the capture region was accomplished. Full scale testing was attempted with raw blood. A bonding failure that causes blood to burst the concentration device prevents final measurement.** These bonding issues should be solved in Phase II with the use of a professional plasma bonding system or a different fluidic pressure source (such as vacuum at the outlet).

2. Phase I detailed progress

The device construction efforts for all tasks have been completed, the blood filtration components have been successfully tested and the functionalization scheme has been developed. Full scale biology testing was accomplished for each of the subcomponents. Integration was also successful. The final subtask of task 3, however, is undergoing troubleshooting and will be considered ongoing work. The following sections detail the results of work performed organized by task.

2.1 Task 1: Nanochannel Concentration

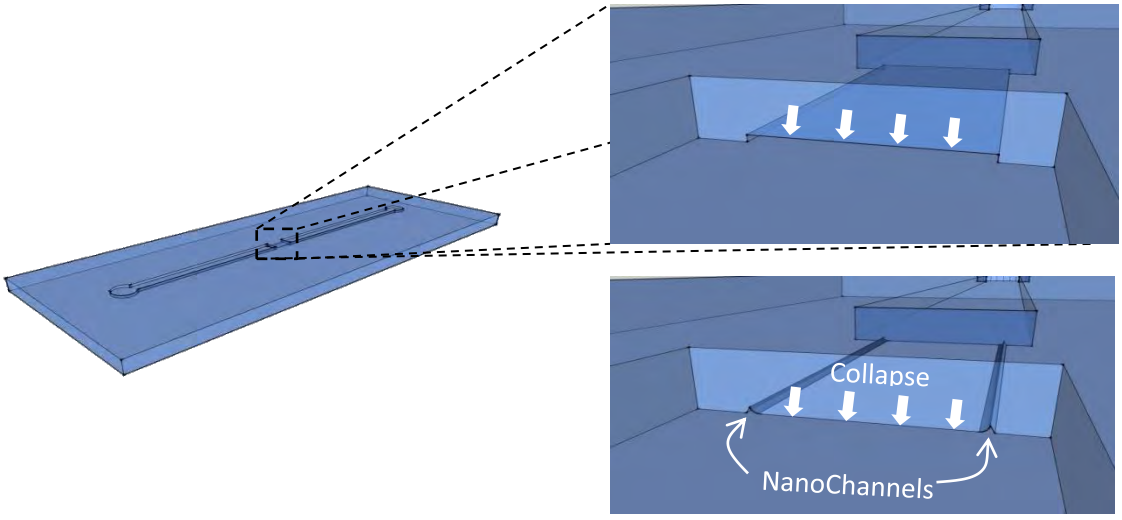


Figure 2. Method of generating nanofluidic collapse channels. Two separate photolithography steps are required to generate the different channel geometries depicted. The initial structure consists of a low aspect ratio channel which collapses leaving two nanochannels for every one original channel.

The method of concentrating the filtered plasma is based on the controlled collapse of microchannel structures to form nanofluidic geometries as shown in Figure 2. As a solution flows through the nanochannel the reduction in channel dimension, which is just an order of magnitude larger than most proteins, will become obstructed and accumulate at the interface of the nanochannel and the microchannel. We plan to take advantage of this phenomenon by placing a sensor array at this interface, thus effectively raising the analytical sensitivity of the device and decreasing the limit of detection.

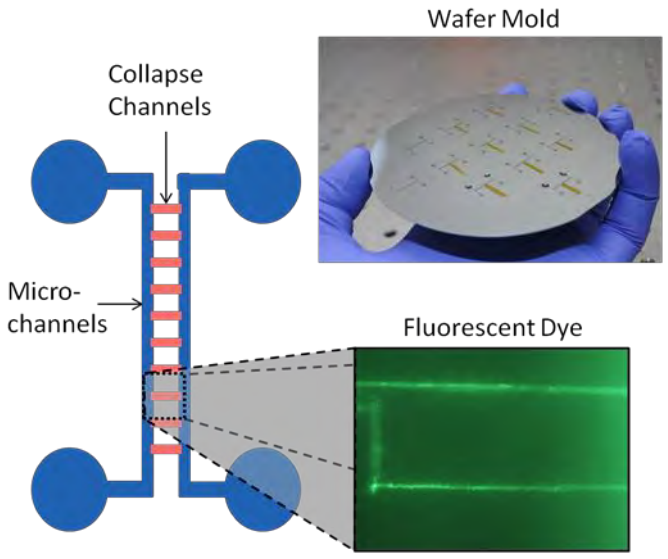


Figure 3: Nanochannel fabrication results. On the left, a conceptual schematic of the nanochannel collapse pattern. The device consists of two microchannels separated by a narrow gap. The nanochannels are patterned in-between and are shown in red. Successful fabrication of channels can be visualized by flowing fluorescent dye through one of the microchannels. On the top right, a successfully fabricated silicon wafer mold is shown.

2.1.1 Nanochannel Fabrication

The fabrication of the device is carried out by performing PDMS micromolding on a silicon and SU-8 master. To make the silicon master, two SU-8 photolithography steps were carried out – the first to pattern the nanochannel elements, and the second to pattern the larger sized channels that serve as the major fluid pathways. PDMS was poured onto the silicon master in a 10:1 elastomer base:curing agent ratio and heated on a hot plate at 150°C for 10 minutes. The hardened PDMS was removed and diced into individual devices. Permanent chemical bonding between the PDMS and glass slides is necessary to form the nanocollapse channels. To carry out the bonding, a laboratory corona treater was used to apply air plasma to the surface of the glass and PDMS, followed by a 2 hour bake on a 150°C hotplate. Following the successful fabrication of the nanochannel collapse devices, visualization can be accomplished by flowing fluorescent dye into the device. Figure 3 shows some results of the fabrication.

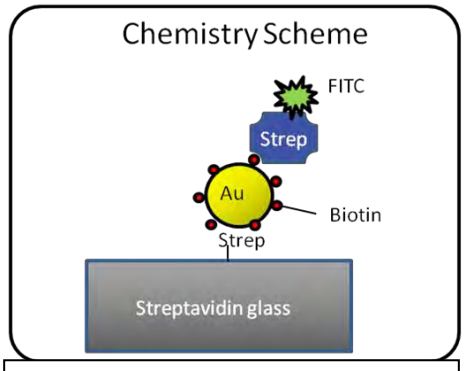


Figure 4: Surface chemistry scheme to perform specific detection of streptavidin.

2.1.2 Specific concentration of streptavidin

Ultimately, a successful test will rely on the output of a sensor that detects levels of biomarkers in blood by selective capture on the sensor surface itself. Before incorporating a sensor into the system, the specific capture and detection of a model analyte should be accomplished to test for proof of concept. In our case, we propose using the nanochannels to enhance biomarker binding by increasing the local concentration. To test this hypothesis, it was proposed to do specific capture of a model biomarker by functionalizing the channel surface in the vicinity of the nanochannels and detect using fluorescence or Raman spectroscopy.

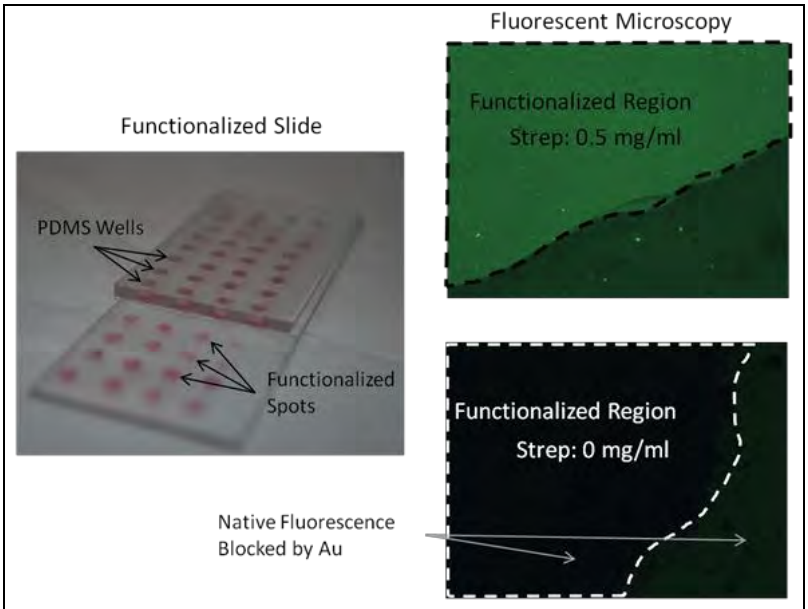


Figure 5: Surface functionalization and fluorescent microscopy of captured streptavidin. On the left, an image of the functionalized slide is shown. The gold nanoparticle-mediated functionalization provides easy visualization of the prepared regions. On the right is a representative example of specifically-captured FITC-streptavidin. The top right image shows 2 hour binding of 3 µl of 0.5 mg/ml streptavidin and the bottom shows the negative control with 0 mg/ml streptavidin.

We had originally suggested performing detection of vasopressin since the technique had been vetted in the Erickson lab and it can be used as a marker for late stage shock. Vasopressin binding via aptamers has undergone significant testing on a variety of devices in the Erickson lab. For example, detection has been carried out on novel SERS structures and using electrochemical biosensor flow cells. **(See Appendix A for more information on vasopressin detection.)** However, upon the recommendation of the grant advisor, we have pursued cooperation with a company (Banyan Biomarkers) that specializes in panels of markers for shock and traumatic brain injury that can be reliably tested by finger stick assay. They use multiple proprietary markers which obviates the need for us to focus on vasopressin, an non-commercialized system. We have therefore decided to move to a more standard and better characterized biotin/streptavidin model system since our development work is focused on the device itself rather than novel surface chemistries. This move has allowed us to make significantly more progress on key areas of development in the allotted time. It will continue to pay dividends in the future, as the important surface functionalization components can be purchased off-the-shelf and are subjected to stringent quality control. Figure 4 shows a cartoon of the surface functionalization

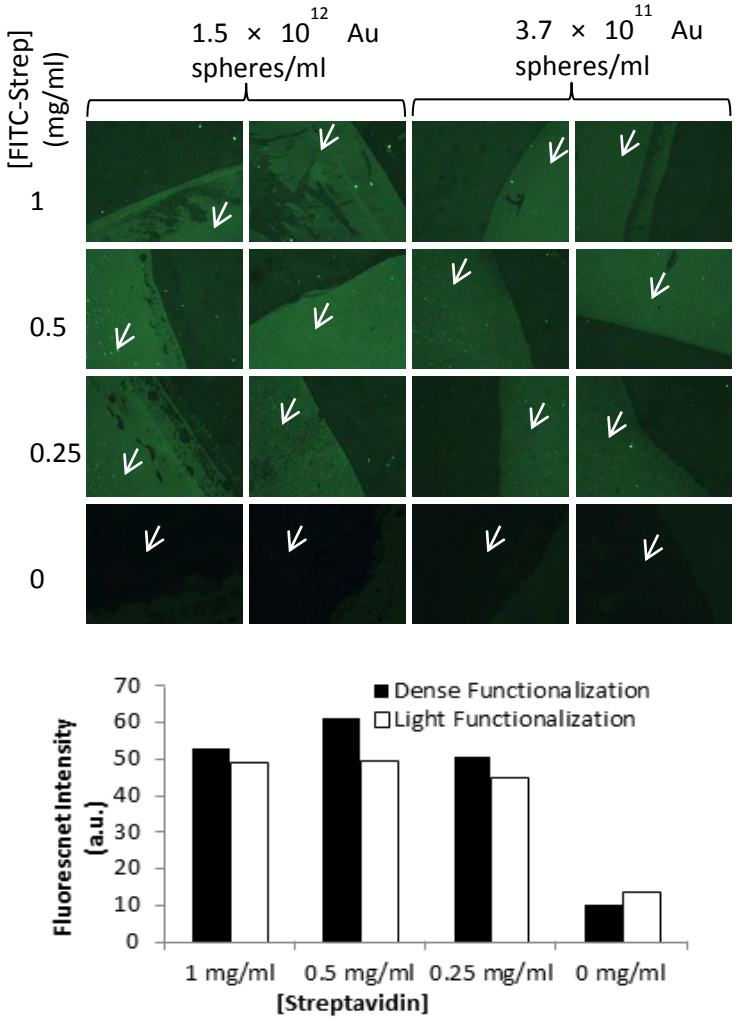


Figure 6: Serial dilution of FITC-streptavidin on two different functionalized surfaces. (Top) Fluorescent microscopy images of the experiment. The white arrows indicate the functionalized regions. (Bottom) Image analysis. Each bar is an average of two measurements. The surface was functionalized with the gold nanoparticle scheme described in the text. Two functionalizations were tested; one using 1.5 ×10^{12} nanoparticles/ml and the other used 3.7×10^{11} nanoparticles per ml. In addition to higher signals, the denser functionalization has the added benefit of producing less background.

chemistry we have selected. Briefly, it consists of streptavidin-coated glass slides and biotinylated gold nanoparticles. Samples spiked with fluorescently labeled streptavidin will be flowed over the surface, which will capture the streptavidin and enable characterization of the nanochannel system. This chemistry was selected for two reasons. First, the gold nanoparticles increase the surface area and should therefore provide enhanced binding kinetics. Second, the gold nanoparticles should serve as an excellent surface for Raman spectroscopy allowing us to perform so-called surface enhanced Raman spectroscopy (SERS). Ultimately label free detection will be carried out by an array of chemical sensors functionalized in a similar way.

Surface functionalization protocol and results: A PDMS sheet with 3 mm through-holes was placed on top of streptavidin-coated slides (purchased from ArrayIt) such that an array of wells was created on the slide. Biotinylated 50 nm gold nanoparticles were incubated in the wells for 24 hours at 4°C in a humidified chamber. After this step, the PDMS wells were washed several times with PBS to remove unbound gold particles. An added benefit of the functionalization scheme is the ability to visually inspect the functionalization sites due to the optical characteristics of the gold particles. The PDMS covered slide and the spots generated during functionalization can be seen in Figure 5.

An experiment to test the functionalization was carried out using two different gold particle concentrations - one with 1.5×10^{12} particles/ml and one with 3.7×10^{11} particles/ml. FITC-streptavidin was incubated on the functionalized surface for 2 hours in three different concentrations (1 mg/ml, 0.5 mg/ml and 0.25 mg/ml) and one negative control (0 mg/ml). Results are shown in Figure 6 which shows average fluorescent intensity of duplicate samples. The denser functionalization method had a better signal strength and lower background noise. The background fluorescence generated by the glass and streptavidin coating was greatly blocked by the gold nanoparticle functionalization scheme, an unanticipated, but important finding. Another important thing to note is that the intensity shows no dose response. This is most likely due to the high concentrations of streptavidin, which saturated the functionalization layer in all cases.

Specific Concentration of Streptavidin from Buffer: The nanochannels are designed to work on serum delivered from the blood filtration column. In order to carry out the functionalization, column packing and sample running in the same device, a variety of channels with several inlets and different flow paths was designed into the device to allow for the different tasks. In this sub-task, functionalization of the region on the upstream side of the nanochannels was carried out using one of the alternative flow paths.

Specific concentration of streptavidin was accomplished by flowing 0.1 mg/ml streptavidin in PBS buffer through the nanochannel concentrators. The channel in front of the nanochannels was functionalized with biotinylated gold nanoparticles as described in the previous sub-section but with some adjustments to accommodate an enclosed microchannel channel rather than exposed glass. To functionalize a microchannel, the biotinylated gold nanoparticles were flowed in using a syringe pump at 2 µl per minute for 1 hour. 50 µl of 0.1 mg/ml of FITC-streptavidin was flowed in at 2 µl per minute (for a 25 minute period) followed by a 1 hour wash with PBS at the same flow rate. Images were taken in a dark environment and measured using

ImageJ. Images of the specific capture of streptavidin show a remarkable increase in local concentration. Image analysis reveals a concentration increase of 13.7 times. A representative image along with a plot of the quantitative image analysis is shown in Figure 7 (left).

2.1.3 Specific concentration from serum

The previous subtask involved specific concentration of buffer-based FITC streptavidin. This task is essentially a repeat of the former task but with FITC streptavidin spiked serum instead of buffer. The functionalization procedure was duplicated on a separate device. Bovine plasma with Na Heparin (Lampire Biological Laboratories, PA) was spiked with FITC-streptavidin to achieve a final concentration of 0.1 mg/ml. The procedure from the previous sub-task to concentrate FITC-streptavidin from buffer was followed here as well.

The concentration effects while using serum are apparent, but not as dramatic as for the buffer case. A variety of factors tied to the use of serum can play a role in decreasing performance. In particular, the mechanical properties of the fluid and the existence of a multitude of other serum proteins could both play a role in decreasing efficiency. We leave optimization of this promising device for serum for future work.

2.2 Blood Separation

For protein-based diagnostic tests performed on blood samples the first processing step is usually removal of the cells to gain access to purified serum. The presence of red and white blood cells and platelets, together composing not quite half of the blood volume, can generate clots and otherwise confound analysis. As described in the proposal, “There have been a number of other microfluidic techniques used to separate plasma from raw blood (e.g. inertial microfluidics, weir type devices and centrifugal systems). These systems are nominally successful but tend to have relatively low plasma capture efficiency (which is extremely important for CLIA waivable devices) or require complex fluidics and powered operation.” In this project, we have used a process first described by Shim *et. al.* that relies on capillary flow through a wetting filtration column that therefore requires no power. The pores of the column are small enough to restrict the flow of red blood cells but easily allow plasma to flow through. The size and surface properties of the microspheres that make up the packed column are the critical features. The size of the beads determines separation efficiency – too large and the red blood cells can pass through – too small and the flow rate is compromised. The surface properties of the microspheres, most importantly their surface energy, determine the ability for water to be driven through the column by capillary effect. The silica particles used to pack the column are not sufficiently hydrophilic on their own to cause capillary flow. A treatment of the column with a blocking buffer, which coats the silica beads with hydrophilic proteins, was therefore required to induce flow.

2.2.1 Fabrication

A variety of column packing methods were tried before a reliable system was found. The three main obstacles were (1) creating a barrier mid-way through the channel that allows liquid to pass but obstructs the beads, allowing us to create a packed column (2) preventing the beads, which are much denser than the liquid, from sedimenting rapidly and clogging the channel inlet and (3) a method for pumping out the suspending fluid and drying the column without destroying the channel itself.

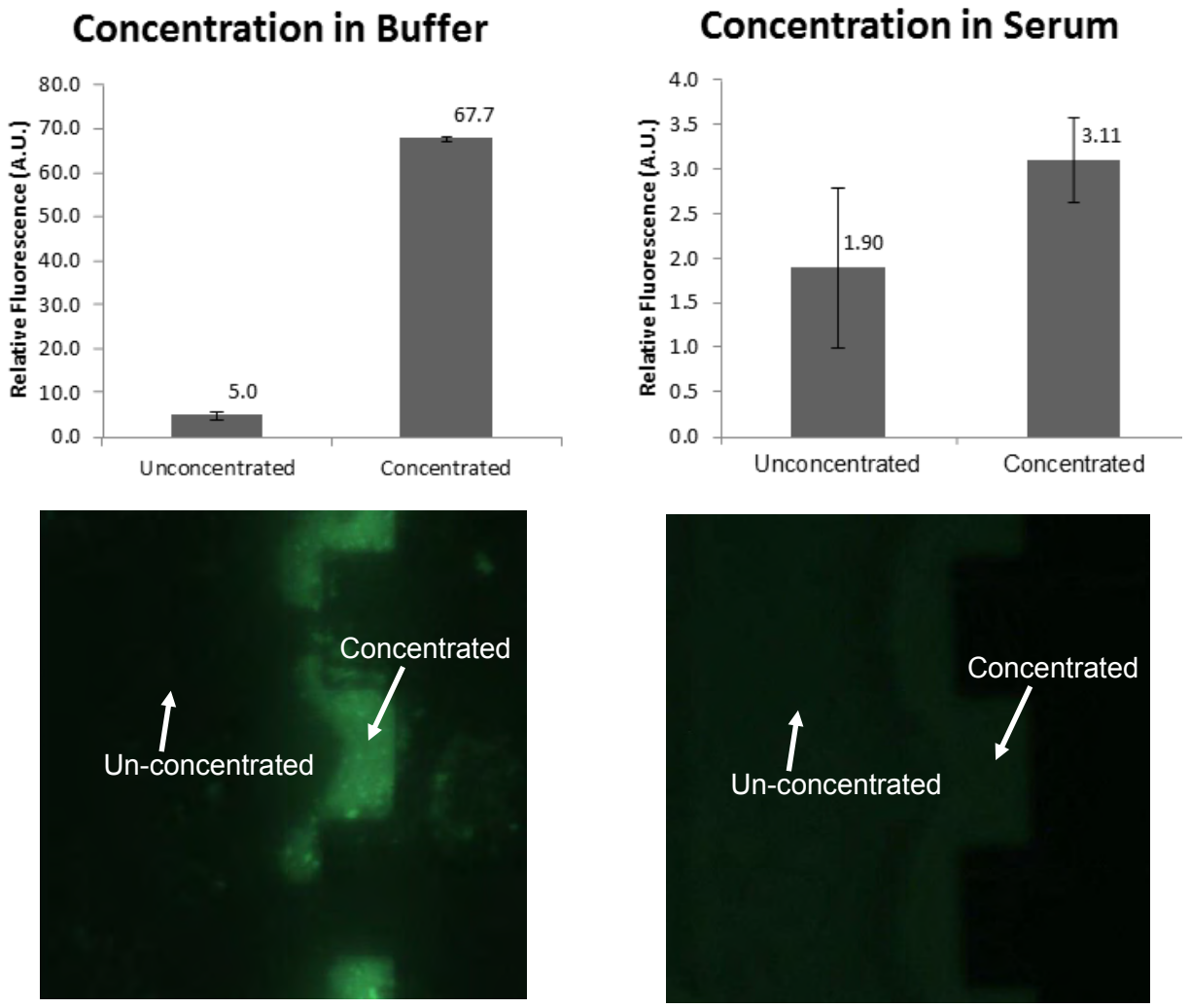


Figure 7: Nanochannel concentration and specific capture of FITC-streptavidin on biotinylated nanogold surfaces. Concentration in buffer has a dramatic effect, increasing the local concentration of streptavidin by nearly 14 times. Serum is also successfully concentrated, but less so – about 1.6 times. Optimization of the geometry and flow conditions will likely improve this value.

To solve (1) and create a packed column of beads, we had originally planned to create a set of microfabricated pillars that extended across the whole channel with gaps between them that were smaller than the diameter of the beads to be used for the column. This method proved unreliable because the channel height had to be high enough to provide sufficient volumetric flow of blood, thus requiring very high aspect ratio pillars. High aspect ratio pillars are notoriously difficult to fabricate by PDMS casting, and so this approach was abandoned in favor of a much simpler method. The elastomeric properties of PDMS allowed us to simply press down on the PDMS with a clamp, causing the channels to become deformed until the microspheres could no longer pass. This method allowed us to reliably create packed columns inside a microchannel. After the column is packed and dry, the clamp can be removed and the column can be used to separate blood.

To solve (2 and 3) we had originally planned on pumping a dilute solution of the microspheres through the microchannel using a pipettor or syringe pump. This method led to a variety of problems. At low flow rates, the beads would sediment en mass, and clog the inlet. At high flow rates, the channel integrity failed, became unbonded and was destroyed. We switched instead to a method of using a very small volume (1 µl) of highly concentrated microspheres that would rapidly enter the column by capillary action. To induce rapid capillary action, the channels were pretreated with 5 seconds of air plasma using a laboratory corona treater. The beads then easily flowed in. To remove the liquid and dry the column, a vacuum was applied to the outlet. The final step was to treat the beads with a blocking buffer to make the surface of the beads hydrophilic for the blood test. 20 µl of Fisher Scientific Starting Block was flowed through the channel and removed again using vacuum again at the outlet. To ensure complete drying, the devices were placed at dry 4°C environment for 24 hours. Another method for

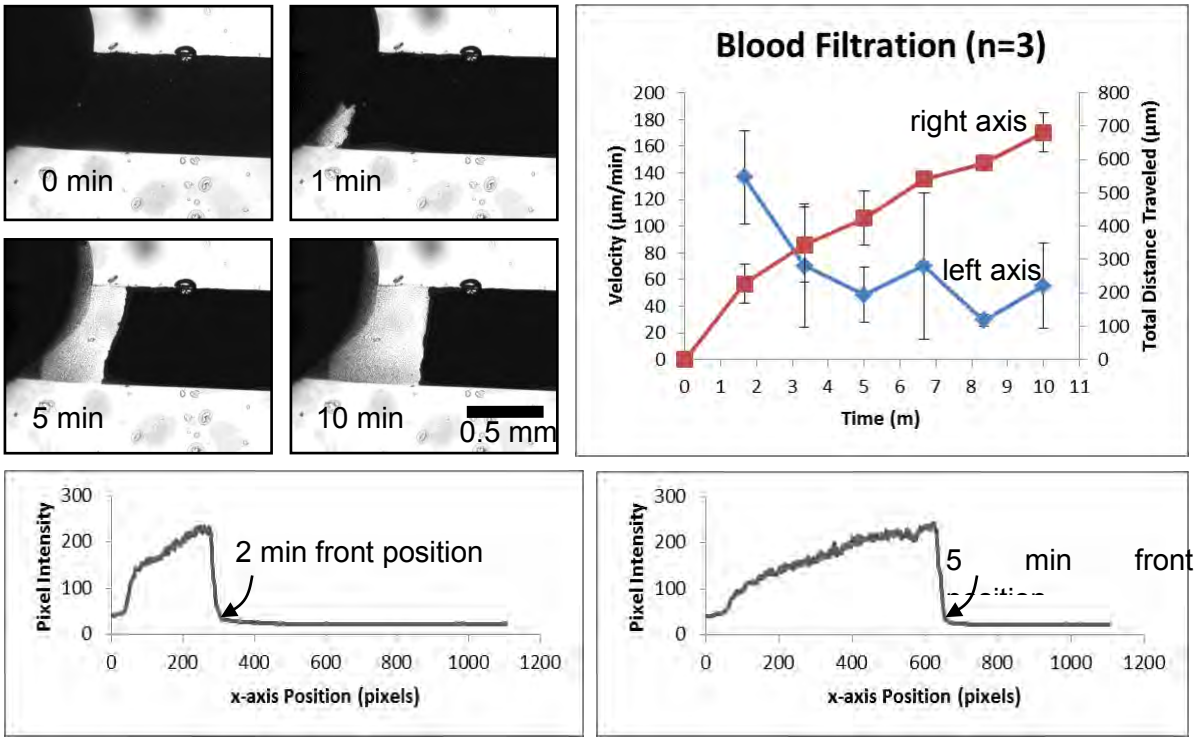


Figure 8: Blood filtration in the microfluidic packed column. On the top left are selected images from one of the experiments using human blood. The column starts out appearing very dark, but then turns white as the filtered plasma progresses to the right. On the top right, an analysis of the moving plasma front for n=3 filtration experiments. On the bottom, pixel profile maps show the location of the front as it moves.

building the packed column relies on centrifugation. This technique was developed for the integrated device and will be discussed in section 2.3.4.

2.2.2 Blood filtration testing

Initial testing of the blood filtration column was carried out using heparinized cow's blood. 10 µl was pipetted into the inlet of the filtration column and progressed well for at least a half an hour. However, after 3 days, the cow's blood no longer behaved the same and would stop flowing

after about 1 minute. Testing on freshly collected human blood from a lanced finger proved much more reliable. To perform a test, 10 µl of blood was collected from a finger prick and loaded into a filtration column. The device was quickly placed under a microscope and a time course of images was taken. The position of the front was calculated using ImageJ to generate a pixel profile plot. A box was drawn to roughly match the channel dimensions and a profile plot was generated. This averages the pixel value in the y direction and plots it as a function of x. Profile maps were taken at different times and used to calculate the velocity of the moving front. See Figure 8 for images and figures.

We should mention that since the fluid is driven by capillary action in a packed bed, the linear flow rate will stay roughly the same for a variety of channel dimensions. The channel can therefore be made taller, shorter, wider or thinner to accommodate the desired volumetric flow rate.

2.3 Task 3: Integration

One of the major advantages of the sampling technology being proposed is the simplicity of the approach. Blood filtration and sample enrichment are carried out within a single flow path with blood filtration operating completely with capillary action. It was found that concentration requires a simple pressure source. This simplicity translates into much easier integration, a ubiquitous difficulty encountered by microfluidics engineers. The two components, the filtration column and the nanochannel concentrator.

2.3.1 Fabrication

Three steps are required for fabricating the integrated device. First, the integrated microfluidic channels must be patterned to create a PDMS molding master. Second, a method for filling the filtration column that is compatible with the nanochannels must be developed. Third, a procedure for functionalizing the entrance region of the nanochannels must be worked out and tested. **All three of these fabrication steps have been successfully completed.**

In order to pack the column, an alternative to the previous methods was needed. The high flow resistance caused by the nanochannels prevented convenient packing by vacuum or pressure-driven flow. Instead, a centrifugation method was developed. A small volume of beads was injected into the column and the entire device was placed into a centrifuge such that the centrifugal force drove the beads against the nanochannel structure. Packing was accomplished at 1000 g for 10 minutes. Greatly improved results were found when 0.5% triton x was added to disrupt surface tension. Columns packed in this way have a much cleaner entrance and appear to be packed more efficiently than previous methods. Moreover, the centrifugation method allows for numerous columns to be packed simultaneously.

2.3.2 Integrated device testing with plasma and whole blood

Upon completing all previous tasks and subtasks, the final integrated device (including blood filtration column, fully functionalized surface with biotinylated nanoparticles and nanocollapse concentrators) was tested with fresh whole blood spiked with 0.1 mg/ml FITC-streptavidin. The devices were prepared as follows. The micro- and nanochannel device was molded from a silicon wafer containing SU-8 features and with some additional hand-placed sections of kapton tape to modify, extend and enlarge the microchannels. The PDMS was bonded to a

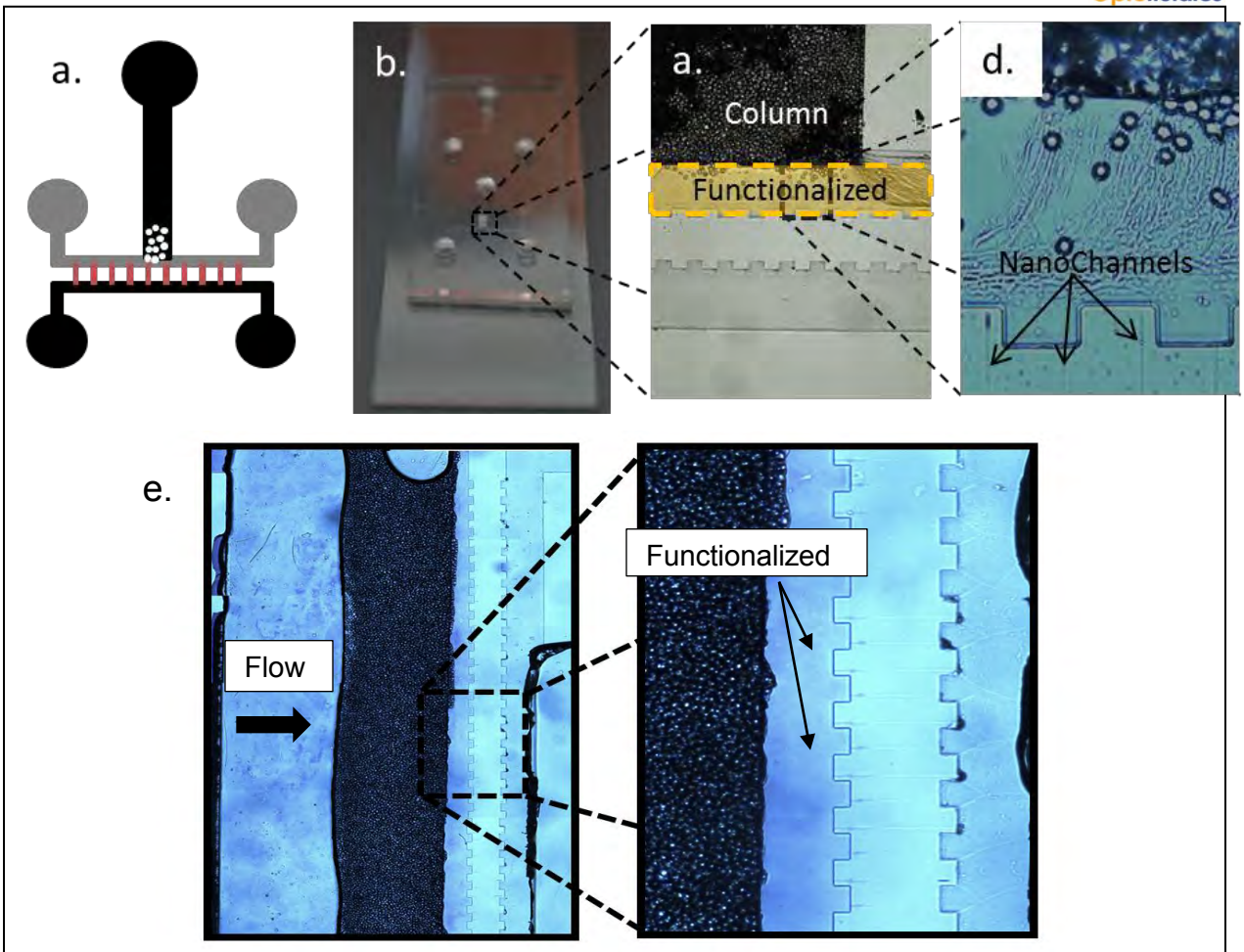


Figure 9: Fabrication of the integrated device. (a) shows a conceptual drawing of the device. The packed column is in the top channel and intersects with the nanochannels below. The gray cross-channel is used for functionalization and is connected to the lower channel through the nanochannels. (b) shows a photograph of the assembled device. (c) and (d) are magnified views showing the column, functionalization region and nanochannels, which are just barely visible in (d). (e) shows a fully prepared device with a functionalized surface, nanochannels and a packed bed of blood filtration beads.

streptavidin-coated glass from ArrayIt using 5 seconds of corona treatment on each piece. After bonding, the low aspect ratio microchannels were collapsed into nanochannel concentrators by applying gentle pressure. This was followed by functionalization with biotinylated nanoparticles. These particles were flowed through the device using the stock concentration of beads (1.5×10^{12} particles/ml) at 500 nl per minute flow rate for 1 hour. After functionalization, the column was packed by adding 2 µl of a 1:1 mixture of 50 mg/ml 15 µm silica microspheres: Starting Block Buffer with 0.5% triton X. This column was packed against the nanochannel by spinning the slide in a centrifuge at 1000 g for 5 minutes. The resulting devices were then dried overnight in a refrigerator.

To run the device, 30 µl of fresh whole blood from a finger stick was spiked with FITC-streptavidin to give 0.1 mg/ml final concentration. This was loaded into the device with a

syringe pump at 2 µl per minute. Initial loading into the device and blood filtration appears to go smoothly. However, after about 5-10 minutes the devices all fail due to a pressure buildup which ultimately detaches the nanochannels from the glass surface. The successful initial progression of the blood and separation, followed by the device failure is shown in Figure 10. A syringe pump was required due to the high hydraulic resistance generated by the combination of the nanochannels with the packed bed of beads. It is hoped that this can be obviated by a low powered pressure source such as a compressed air cartridge, an actuated pouch or a pierced vacuum container. Approaches to eliminate the failure mode include improved bonding between the PDMS and the glass slide using a vacuum oxygen plasma system or using vacuum at the outlet rather than positive pressure at the inlet.

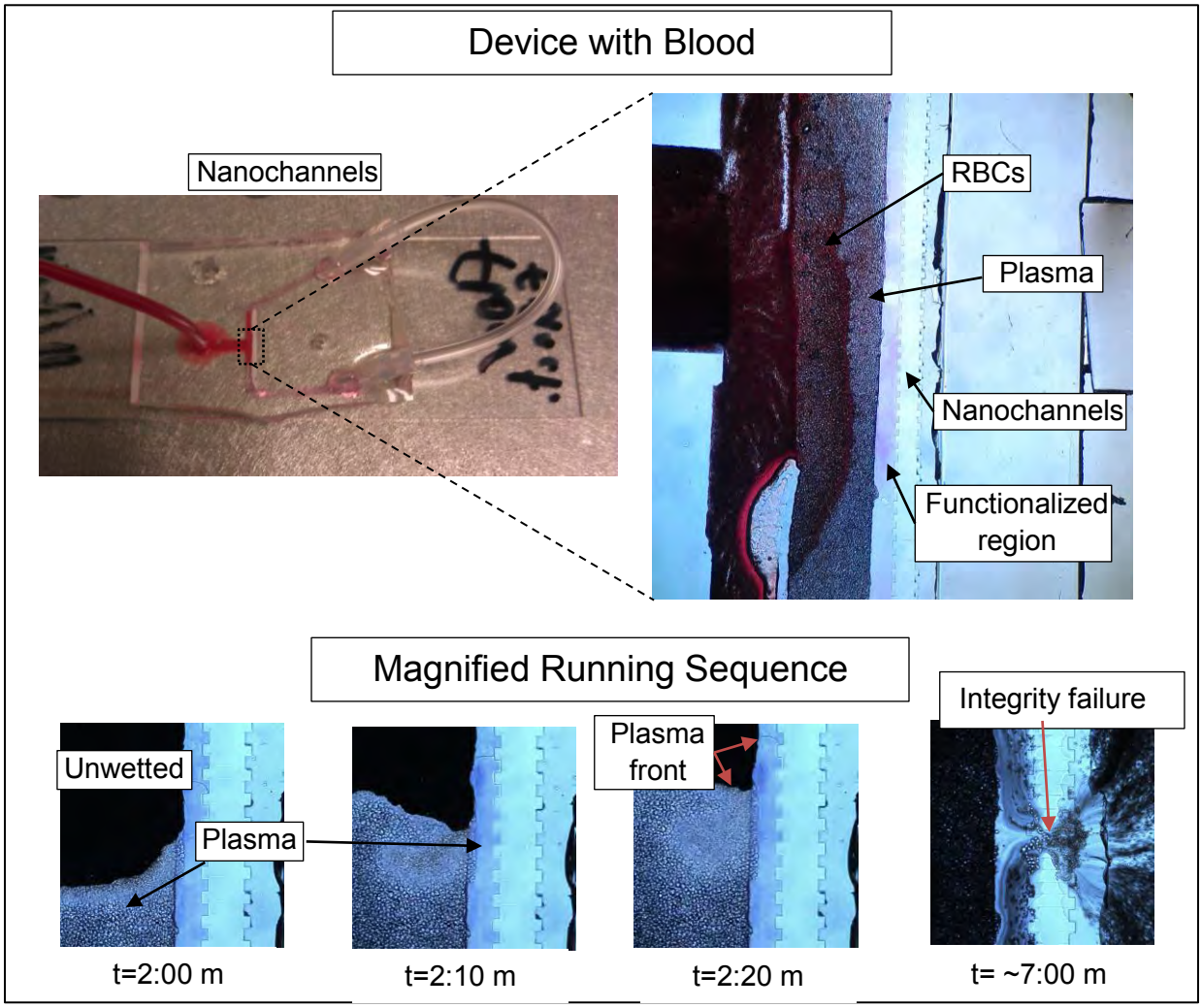


Figure 10: Fully integrated device testing with fresh whole blood spiked with FITC-streptavidin. The top images show the device with blood being loaded in. The bottom images show a sequence of images taken during the experiment. The first three show the movement of the plasma front. The fourth shows the failure mode after about 7 minutes.

2.4 Additional work – construction of chip packaging

Though not a requirement for this Phase I project, the development of chip packaging was seen as an important step towards product development. A proper device housing can facilitate sample delivery and aid in the early identification of problems that are more easily addressed during initial development stages. The housing itself also helps to improve ruggedness and protects the critical parts of the device from getting dirty, a particular problem for devices made with microchannels, which can clog from atmospheric dust or when trying to perform quantitative fluorescent microscopy, which is sensitive to dust, fingerprints, scratches, etc.

Using a 3D printer, two working prototypes of field chip packing were created and are shown in Figure 9. The housing was designed around a microscope slide. The device can either be snapped together or secured using screws. Future versions may be dramatically reduced in scale since the essential components of the chip are less than a square centimeter. We can envision the future device, including packaging, to be about the size of a USB thumbdrive.

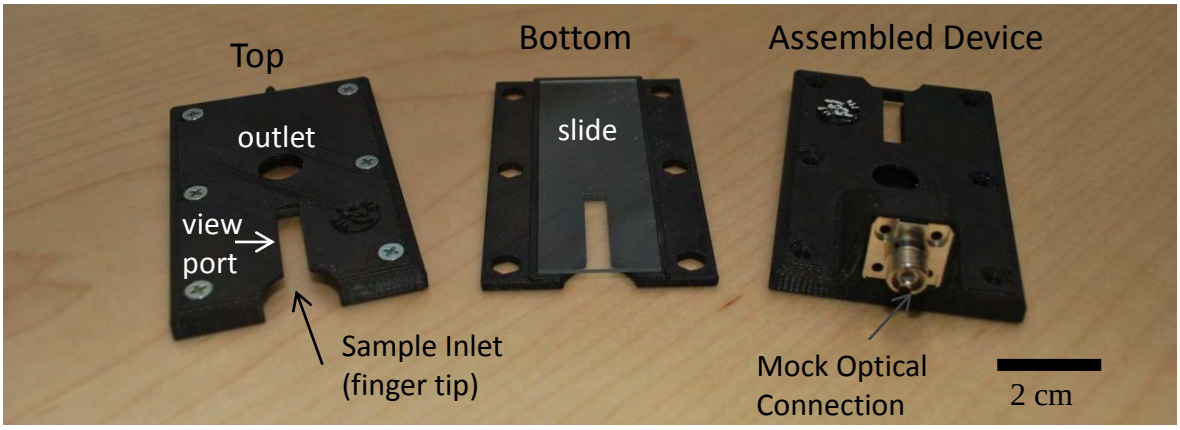


Figure 11: Working prototypes of the field chip packaging. The chip has an inlet, where a patient can place his finger to transfer the drop of blood to the sample collection port. For this early pacakaging, there are also view ports above and below the device components located on the slide. These windows allow for microscopy to be carried out. A mock optical connection is shown but non-functional.

3. Conclusion

This Phase I DARPA project to develop the Molecular Medic, a point-of-injury sample processing technology has made great strides towards creating a system capable of taking a drop of blood from a finger stick and producing purified enriched plasma that is ready for detection on a sensor platform. There were three main tasks in this Phase I SBIR project. The first was to develop a preconcentration device capable of accepting plasma and enriching the local protein concentration in order to raise the analytical sensitivity of the device and decrease the limit of detection. The second task was to develop a blood filtration device capable of accepting blood and producing plasma by capillary flow through a packed column. The third and final task was integration of the two components and included full scale biology testing.

In the past six months, we have designed and constructed all of the components of the device encompassed within tasks 1 and 2 and integrated them into a single micro/nano-fluidic platform

task 3 despite the aggressive time schedule and limited budget. The nanoconcentrator (task 1) consists of nanochannels rapidly prototyped in PDMS using a novel technique developed in our founder's lab at Cornell and which is being licensed by Optofluidics. These concentrators were tested using a model fluorescently labeled protein (streptavidin) and can increase the local concentration by an order of magnitude. It is designed to accommodate a nano-scale optical sensor array whereby the individual sensors are placed in the high concentration "cloud" generated by the concentrators, thus effectively raising the anylitical sensitivity of the device. The blood filtration unit (task 2) consists of a packed bed of carefully sized silica microspheres, chemically treated to rapidly wick blood. As the raw blood progresses through the device, the red blood cells are obstructed and are outpaced by the more rapidly moving plasma. This filtration unit was successfully tested with raw human blood collected from a finger stick without the need for anticoagulants. It produced purified plasma through capillary effect alone. Integration of the two components (blood filtration and plasma concentrator) was carried out successfully for task 3. Testing with whole blood has been initiated but is ongoing due to unforeseen challenges with device bonding integrity. The high hydraulic resistance produced by the nanochannels, combined with the blood filtration column and the use of viscous blood has necessitated the use of a pumping source. The use of buffer causes no difficulties with either of the two components alone, but once integrated the devices fail. It is expected that the bonding integrity can be dramatically improved with the use of a professional oxygen plasma system. In addition to the progress made on tasks 1, 2 and 3, additional work was carried out developing a packaging system for the device which improves ruggedness and provides a means for optical connections.

We believe the great strides taken in this Phase I will produce an important technology that can be incorporated into point of care devices dramatically improving their performance. The developed system can be tailored towards integration with a sensing platform such as the one developed by the Erickson lab at Cornell. Moreover, the envisioned system will be broadly applicable beyond the military, for example for first emergency responders and for rural hospitals or general practitioner's offices.

Appendix A: Previous work detecting vasopressin

1. Vasopressin detection on an electrochemical sensor.

An electrochemical sensing platform was developed in the Erickson lab and shown in Figure A1. It consists of PDMS microchannels on a gold-patterned silicon wafer. In one experiment, the prepared vasopressin solution was introduced into aptamer-conjugated CNT pattern through tubing in microfluidic biosensor system. The change of electrochemical signals from binding between aptamer and vasopressin was detected in Electronic Probe Station Instrument. A calculated limit-of-detection is 42.95 pM, with detection sensitivity of 100 pM. The vasopressin detection results are summarized in Figure A2.

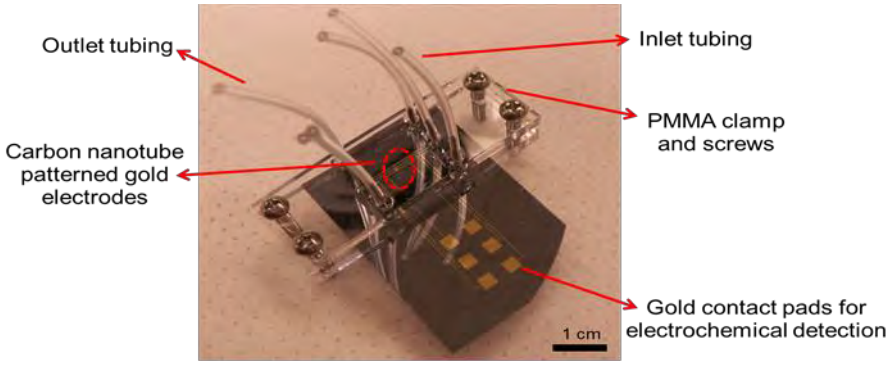


Figure A1. Picture of aptamer-based microfluidic biosensor.

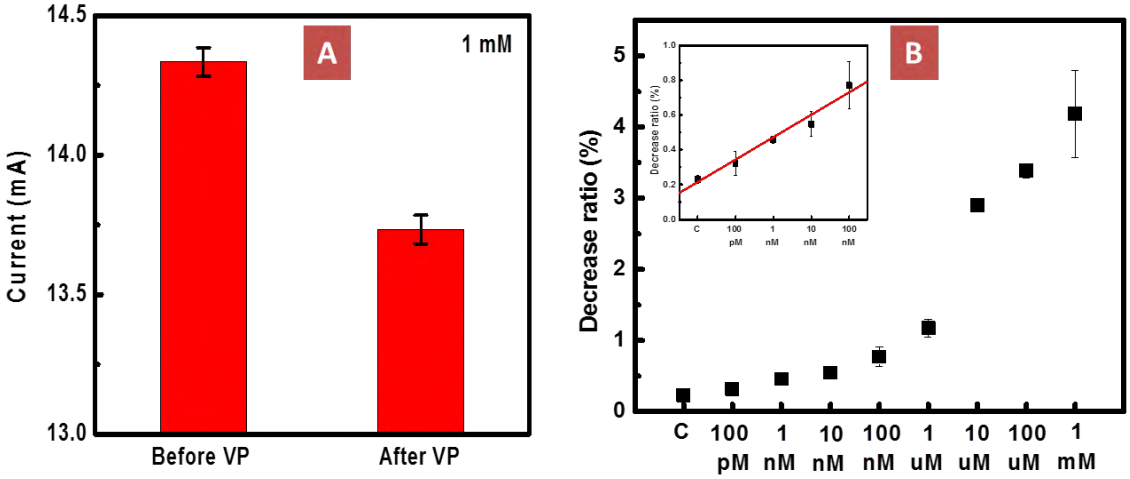


Figure A2. Vasopressin detection results: (A) Electrochemical signals before and after vasopressin detection; (B) Sensitivity measurement of vasopressin detection.

2. Vasopressin detection using a novel SERS substrate

Figure A3 shows preliminary data showing the concentration and detection of vasopressin. Details of these experiments are available from the Erickson 2010 Biosensors & Bioelectronics paper which is available upon request. However briefly in this study vasopressin and BSA (Bovine serum albumin) were purchased from Sigma and a fluorescein labeling kit was used to functionalize the vasopressin with a SERS-active dye. The aptamer sequences were purchased from Integrated DNA Technologies. Raman measurements were made using an inVia Raman microscope spectrometer coupled to a Leica microscope by focusing the excitation laser on the nanotube arrays located in the CCMR at Cornell. The 488 nm line of an Ar^+ ion laser was used as optical excitation source and the scattered signal was collected by a Peltier-cooled CCD detector.

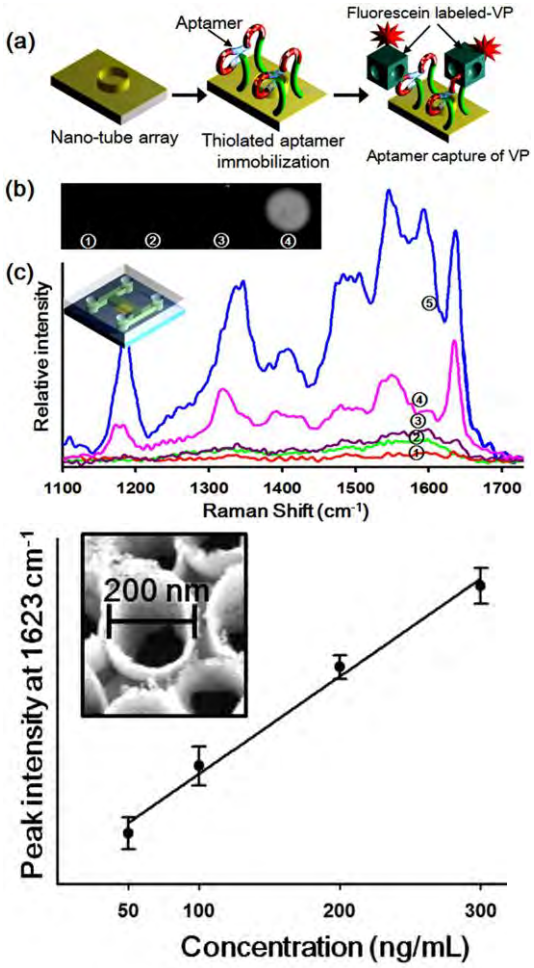


Figure A3. Upper Image: (a) Schematic of the aptamer-VP recognition binding reaction. (b) Fluorescence images of FITC-labelled aptamer-VP reaction on 200 µm patterned spots under the following conditions (1) background control sample of bare nanotube arrays, (2) after immobilization of the aptamers, (3) after aptamer-BSA reaction (negative control) and (4) after aptamer-VP reaction (positive control). (c) SERS spectra of aptamer-VP reaction corresponding to the first 3 cases described shown in image (b) and (4) for 200 nm Au nanotube arrays and (5) Au/Ag/Au nanotube arrays. Lower Image: Concentration response curve.

As is shown in this figure, after immobilization of the aptamer onto the external layer of the SERS substrate, the FITC-labeled vasopressin in PBS buffer solutions were introduced via the inlet port on the microfluidic device. After the reaction was completed, the excitation laser was focused on to the nanotube substrate and the spectrum recorded over a quick integration time of 15 s. To observe the reaction specificity, the fluorescence intensity of each spot was analyzed. As can be seen in curve Figure A3 Upper image part (b), strong fluorescence signals were detected in the positive sample at which the FITC-labeled VP had been added. In the negative control experiment, almost no fluorescence signals were detected at the spots of the FITC-labeled BSA. **These results suggest that the aptamer binding assay is a reliable and specific method for detection of vasopressin.** Figure A3c shows the SERS spectra collected for a number of different controls and SERS substrate

designs. The lower image shows the linear response of the detection assay down to 50 ng/mL. Since the aptamer assay has already been demonstrated and the interfacing of the nanofluidic collapse device with the SERS active substrate will have already been demonstrated in Subtask #1 we do not expect major difficulties in accomplishing this subtask.